CHARACTERIZATION OF THE GENETIC STATUS OF POPULATIONS OF RED JUNGLEFOWL[1]

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(*With one text-figure*)

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The native range of the red junglefowl (*Gallus gallus*) in Southeast Asia and the Indian subcontinent has been the focus of studies of domestication of this species that became the foundation of a worldwide multi-billion dollar poultry industry. Such studies must be based on a thorough understanding of the behaviour, ecology, and biogeography of current as well as past populations. Although red junglefowl are considered abundant both in captivity and in the wild, and have usually not been accorded any particular conservation concern, almost all populations show morphological characteristics suggestive of past hybridization with domestic birds, and indeed pure genomes may prove to be now extinct in the wild. However, one captive population still shows two morphological characteristics considered to be indicative of genetic purity: (1) an annual moult to a dark/black eclipse plumage in the male, and (2) complete absence of combs in females. Preliminary molecular genetic studies of these birds indicate that they are more distinct from other captive strains than the latter are from domestic chickens. These captive birds may thus represent the last pure red junglefowl genomes. This paper establishes criteria for the judgment of genetic purity, in the hope that colleagues across southern Asia will assess local wild populations to develop an accurate picture of the genetic status of this species across its range.

Introduction

Red junglefowl (*Gallus gallus*) represent the ancestor of the most important bird species in economic terms — chickens, which constitute the basis for the multi-billion dollar poultry industry. Although wild red junglefowl are generally not considered to be of any conservation concern, studies of historical and recent museum specimens suggest that wild genomes may be critically endangered or even extinct in the natural state (Peterson and Brisbin 1998). One captive population (hereafter referred to as the JFW strain), however, has been kept in genetic isolation for more than three decades (Brisbin 2000, Hawkins 2001), and shows morphological characteristics which may offer unique insights into the history and current status of the red junglefowl.

To approach these questions of genetic purity, however, requires a thorough knowledge of the morphological, ecological, and genetic characteristics of both the present-day and historical junglefowl populations. Traditionally, such studies have been based on examination of the phenotype, particularly as manifested in studies of captive birds and museum specimens (Delacour 1977). More recently, quantitative studies of museum specimens have revealed patterns of successive loss of characters presumed to indicate genetic purity in wild populations

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(Peterson and Brisbin 1998). The picture, nevertheless, remains incomplete, with only fragmentary survey information for wild populations and captive strains, as well as poor understanding of phenotypic markers used for such surveys (Peterson and Brisbin 1998). Additional tools drawn from molecular genetics and zooarchaeology have yet to be applied to this question; the purpose of this paper is to initiate the collection of such information, as well as to stimulate broader surveys of wild populations and captive strains.

Morphological Characteristics of Pure Wild Red Junglefowl

Our evaluation of the morphological characteristics of pure red junglefowl is based on features considered characteristic of genetically pure wild red junglefowl (Delacour 1977). Critical characters include (1) a complete moult to an overall dark/black "eclipse" plumage by the male following the breeding season (generally June-September), and (2) complete absence of a comb in the adult female. Other traits are cited as distinguishing pure wild junglefowl (Nyunt 1993) but have been generally found to be less reliable: (1) slender, dusky tarsi of wild birds are shared by several domestic forms (Smyth 1990), (2) longer spur-lengths than domestics (Nyunt 1993) has been discounted by our preliminary studies (Brisbin and Peterson unpubl. data).

We have surveyed informally the occurrence of male eclipse plumages in captive red junglefowl in North America, as well as in 351 skins of adult wild junglefowl in 19 museum collections (Peterson and Brisbin 1998). These surveys suggest that the JFW population is the only North American red junglefowl captive population in which all birds consistently show the two characters listed above. The museum surveys also indicated, on the basis of the occurrence of male eclipse plumages, that genetically pure red junglefowl may also be extinct or critically endangered in the wild. This trait apparently disappeared from extreme Southeast Asia and the Philippines (if the latter populations are indeed native) prior to the mid-late 1800s, and from the Malaysian region in the 1920s. Two recently examined skins indicate the survival of eclipse plumages on Hainan Island until the 1930s (Beijing Zoological Institute 01587, 01586). The last museum specimens showing male eclipse plumages were taken from north-central India in the mid to late 1960s (Peterson and Brisbin 1998), exactly the time and place that the founders of the JFW population were brought out of the wild as part of an exotic gamebird propagation and release program of the U.S. Fish and Wildlife Service (Bohl and Bump 1970).

History of the JFW Red Junglefowl Population

The morphology and geographic distribution of extant subspecies of red junglefowl have been described and analysed in detail for decades (Delacour 1977). Studies of the JFW population, however, have raised serious questions concerning the morphological and genetic characteristics of pure red junglefowl (Peterson and Brisbin 1998). This small captive population is now being maintained by a consortium of private aviculturists in the southeastern United States (Brisbin 1996, Brisbin 2000, Hawkins 2001), and may now represent the only source of genetically pure red junglefowl in the wild or in captivity (Peterson and Brisbin 1998).

The JFW population was established from a small but undocumented number of founders captured in north-central India, in the vicinity of Dehra Dun, in the mid to late 1960s (Bohl and Bump 1970). Descendents of the wild founders were distributed to propagation centres in eight states in the southeastern United States, where over 6,000 birds were produced and released in natural habitats throughout the

region. Over the years, however, there has been no indication of long-term survival of free-ranging birds in any of the releases, and the program was terminated in the late 1960s.

At this time, a second founder population of 50 chicks was taken from the Bowen's Mill hatchery, near Fitzgerald, Georgia, USA, and moved to the University of Georgia's Savannah River Ecology Laboratory, near Aiken, South Carolina. They were maintained in captivity and used in behavioural and ecological studies for several years (Brisbin 1969). From the early to mid 1970s through 1997, the entire JFW population was maintained in random pure captive propagation by a private aviculturist in Tuscaloosa, Alabama, with an annual pre-breeding population size of 10-20 adults of approximately equal sex ratio. Morphological and behavioural characteristics did not change appreciably from those of the original birds nearly 30 years earlier.

This character stability has been particularly true regarding the extremely wary and flighty nature of the JFW birds, which has persisted in spite of continuing efforts to imprint and tame incubator-raised chicks. These observations confirm the findings of earlier behavioural studies that indicated little modification of their flighty nature in foster-rearing under tamed hybrid "zoo-type" red junglefowl hens (Brisbin 1969). Foster-reared birds, upon attaining sexual maturity, showed little tendency to integrate into the social hierarchy of the resident, free-ranging flock of hybrid junglefowl. They kept to themselves, and eventually dispersed into neighbouring wooded habitats and disappeared.

In 1998, 65 hatch-year JFW birds were removed from the collection in Alabama and distributed among several private aviculturists in Georgia and South Carolina with a dozen or so adult breeders being retained in the Alabama collection. The population is thus dispersed now among experienced breeders, who are working together to ensure the continued existence of documented genetically pure birds in several captive sub-populations.

Molecular Genetic Studies

The unique nature of the JFW population suggested the importance of a molecular genetic characterization of these birds, particularly in the light of recent efforts to use molecular methods to identify the wild ancestors of domestic chickens (Siegel *et al.* 1992, Fumihito *et al.* 1994, 1996). Though preliminary, the results of our first steps in this direction are reported below.

Mitochondrial gene sequences were derived from PCR amplification products obtained from feather samples. Samples were taken from two JFW individuals, a domestic chicken of undetermined breed, and two domestic/feral bantam chickens from a specially-bred flock at the Savannah River Ecology Laboratory (Brisbin 1993). Samples were also analyzed from two captive zoo junglefowl with morphological characteristics suggestive of domestic contamination, from the Riverbanks Zoo, Columbia, South Carolina; these birds were direct descendants of the free-ranging "red junglefowl" formerly maintained at the San Diego Zoo (Collias *et al.* 1994). Outgroups for phylogenetic analyses included similar samples from a green junglefowl (*Gallus varius*), Malayan peacock-pheasant (*Polyplectron malacense*), and Bornean peacock-pheasant (*P. schleiermacheri*), all from the collections of the New York Zoological Society.

We sequenced 1011 base pairs from two regions of the mitochondrial DNA (mtDNA) genome: (1) the relatively conservative 16S ribosomal gene, and (2) a portion of the more variable, protein-coding, cytochrome *b* gene. We used published primers based on the domestic chicken sequence for PCR amplification, and PCR products were sequenced directly on an ABI automated sequencer.

The 16S sequence data were invariant in

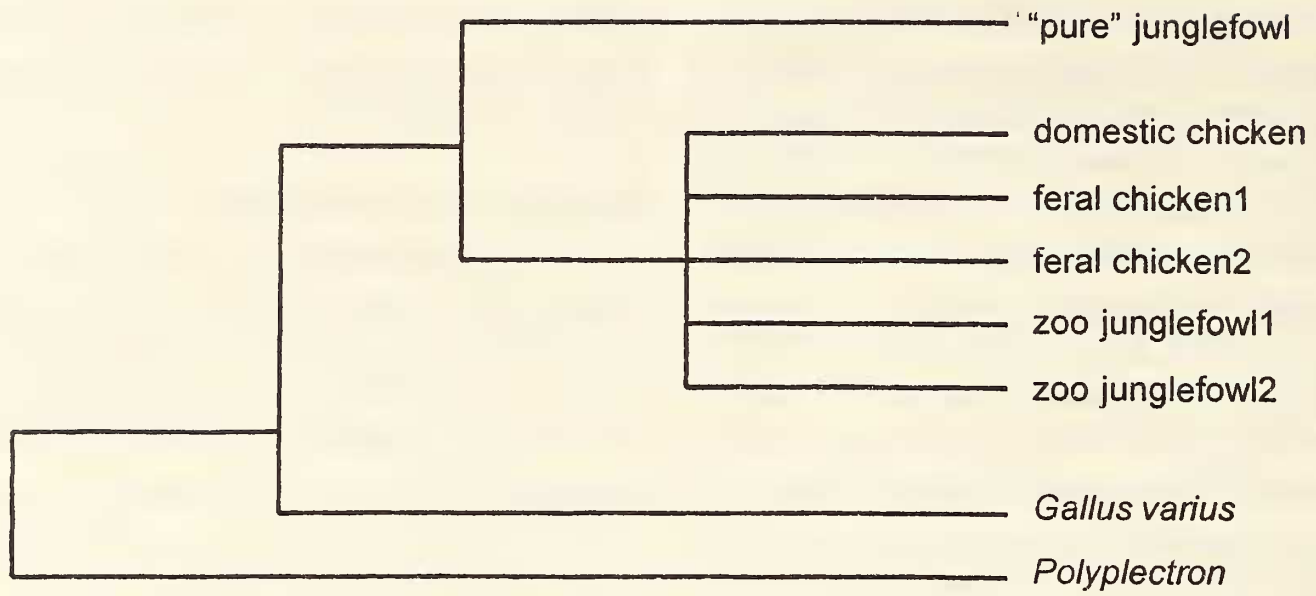

Fig. 1. Diagrammatic representation of results of preliminary phylogenetic analyses of mitochondrial DNA sequence data in junglefowl, chickens, and related pheasants. Feral chickens were taken from a special flock developed at the Savannah River Ecology Laboratory; individuals 1 and 2 represent different specimens of each form

all four junglefowl and the domestic chicken. Sequences of the green junglefowl and the two peacock-pheasants were distinct. This result is more or less typical for this highly conserved gene region. Cytochrome *b* sequences, considering the small number of red junglefowl sampled, were fairly variable. The same sites were variable in both the JFW and zoo junglefowl groups. The two JFW individuals had identical haplotypes, which was not surprising, considering the bottlenecks of low population numbers in the history of this group.

We analyzed these data phylogenetically, treating individual bases as unweighted and unordered characters. A single, most parsimonious tree placed the JFW birds basal to the two zoo junglefowl and domestic chicken (Fig. 1; consistency index 0.86). Genetic distances between the zoo junglefowl and domestic chickens were shorter than between the zoo junglefowl and the JFW birds. Most importantly, the JFW haplotype included two sites that were plesiomorphic when polarized by outgroup comparison, suggesting that this population does not share the common ancestry that is shared by the domestic chickens and the zoo junglefowl. Still, caution must be used in interpreting this information, given the small sample sizes available.

We sequenced additional mtDNA from a single JFW male to parallel prior studies of chicken and junglefowl molecular genetics, focusing on the 392 base pair portion of the noncoding control region studied by previous investigators (Fumihito *et al.* 1994, 1996). The work of these authors, however, lacked samples from the western extreme of the species' distribution in India. Our resulting JFW sequence showed 2% divergence from the published Barred Rock domestic chicken sequence (Fumihito *et al.* 1996). The JFW sequence fell within a clade that included all domestic sequences, and grouped broadly with Thai red junglefowl and Asian domestics, but was more distinct from western domestics. If the JFW sample had fallen outside of the domestic clade, the Thai-origin model (Fumihito *et al.* 1994, 1996) would have been supported. Rather, our results failed to support the conclusion of a Southeast Asian origin of domestic chickens.

Our molecular data do not exclude a model of Indian origin of domestic chickens. Here, the DNA composition of the Southeast Asian junglefowl used in previous studies (Fumihito *et*

al. 1994, 1996) would be interpreted as showing the effects of hybridization with feral or domestic village chickens in that portion of the species' distribution. Under this scenario, Fumihito *et al.'s* Indonesian genotypes could possibly represent the original Asian red junglefowl types. To support this alternative model, it would be necessary to show that museum specimens of birds collected earlier in Southeast Asia have different mtDNA types from those "red junglefowl" now found there, and that Indian red junglefowl have high mtDNA diversity.

Taken together, the above findings have important implications for understanding chicken domestication. They particularly emphasize the importance of documenting the characteristics and history of populations from which samples are taken for DNA analysis. In the case of Fumihito *et al.* (1994, 1996), "wild" Southeast Asian junglefowl profiles were based on samples taken from zoo birds and other populations of unknown provenance. Personal observations by ILB, however, suggests that the wild behaviour of pure red junglefowl, such as the JFW birds, prevents them from being maintained on exhibition in most public zoo collections, where stress would be extreme. Furthermore, the external morphology of all zoo junglefowl we have observed fails to conform to the characteristics of pure wild genetic ancestry (Delacour 1977). Thus, without further information, the Tama Zoological Garden's "Thai red junglefowl" used in the molecular studies (Fumihito *et al.* 1994, 1996) must remain suspicious as possibly showing the results of past genetic contamination. Additionally, birds described by the same authors as "gifts from the Department of Forestry of the Thai government" could have been obtained from near villages, where hybridization could have occurred even in the free-ranging state. In fact, our studies of museum specimens (Peterson and Brisbin 1998) suggest that morphological traits indicative of pure wild ancestry disappeared from these areas over 60 years before the sampling for that study. Hence, there is a real possibility that the similarity of molecular characters of these birds to those of domestic birds results from past hybridization, rather than being indicative of their status as the progenitor of the domestic birds.

Implications for Chicken Domestication

An important application of our findings is in the interpretation of ancient artifactual depictions of birds. Regarding traits indicative of pure wild stock (Delacour 1977), we are unaware of any representation of a male *Gallus* in what could be the dusky eclipse plumage, lacking the elongated bright-coloured neck hackles. The absence of such representation suggests either that this trait was lost early in the domestication process, or perhaps that its drab appearance was not considered worthy of depiction by ancient people. Similarly, with one possible exception, we are unaware of any representation of early female *Gallus* lacking a visible comb and facial wattles. An early Egyptian "chicken hieroglyph" depicts "the chick . . . but never an adult bird" (Zeuner 1963), which is the only possible exception. Given that the wild junglefowl would be combless in the adult hen (Delacour 1977), we suspect that this hieroglyph may actually depict a combless hen such as those of the JFW strain.

An important question is how could ancient people with limited facilities and skills for husbandry have managed to tame junglefowl to produce a captive and later domestic population, from such a wild and wary bird? Even early imprinted and hand-reared chicks of wild stock would have been extremely difficult if not impossible for ancient people to raise and breed successfully in full captivity or semi-confinement (Brisbin 1969, Bohl and Bump 1970). A more likely ancestor of domestic chickens would be more docile in disposition, show a prominent comb in hens, and might lack an eclipse plumage

in the male. The discovery of such a population would leave unanswered the question of the status of populations of India, including the JFW birds.

Hence, our studies raise the possibility that the JFW population may represent a well-differentiated group within red junglefowl, possibly a cryptic species, that may not have been involved in the domestication of chickens. Such a scenario has important implications both for understanding the biogeography and ecology of chicken domestication, as well as for the conservation of populations of captive and free-ranging red junglefowl. Perhaps the most parsimonious conclusion, however, is still that of the genetic purity of the JFW population, and the contamination of the rest of the populations of this species.

Future Directions

Clearly, continuing the pure captive propagation of the JFW population remains a priority. As available numbers and natural mortality permits, we are preparing a complete age series of study skins and skeletal material from these birds to permit thorough molecular and phenotypic comparisons. Several other research avenues remain, however, including the following:

1. Broad molecular surveys to establish phylogenetic patterns with much-improved detail, presently under development.
2. Broad phenotypic surveys to document geographic pattern of variation in critical characters, particularly as regards detection of previously unappreciated geographic breaks.
3. Limited hybridization and backcross experiments to assess the genetic basis for the phenotypic markers described above. Such experiments have now passed to the second generation of backcross of hybrids to JFW stock, providing a known-purity standard for evaluation of phenotypic markers.
4. Surveys of phenotypic and molecular characteristics of wild and captive populations of red junglefowl. This step is particularly critical in eastern and north-central India, where the probability of survival of pure stock is highest; some indications exist of possibly "clean" captive and wild populations in some remote areas of India (G. Das, pers. comm.), making this step of utmost importance.

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